Albumin–Lipid Interactions: Prostaglandin Stability as a Probe for Characterizing Binding Sites on Vertebrate Albumins†

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ABSTRACT: We determined the effect of vertebrate albumins on the stability of several physiologically relevant prostaglandins. All naturally occurring prostaglandins with a β-hydroxy ketone group decomposed by first-order kinetics, dependent on the albumin concentration in 0.1 M, pH 7.4, buffer at 37 °C. Even subphysiological levels of albumin (1–20 mg/mL) significantly reduced the stability of these compounds in vitro. The prostaglandins with a β-hydroxy ketone responded to albumin in the order of their intrinsic stability; namely, less stable compounds were more susceptible. The destructive effect of albumin was nearly maximal at a 1:1 mole ratio of albumin (20 mg/mL):prostaglandin (100 μg/mL). Albumin had no destructive effect on prostaglandins without a β-hydroxy ketone. Albumins from different vertebrates varied in destructive severity, but all were effective. Near neutrality, in the absence of albumin, decomposition of E-type prostaglandins was practically suspended at the dehydration stage. In the presence of albumin, dehydration was accompanied by rapid isomerization reactions (e.g., $PGA_1 \rightarrow PGB_1$) that occur only at an elevated pH. The results suggest that albumin sequesters prostaglandins to one principal binding site and exposes them to its associated highly alkaline microenvironment. This results in a uniform and predictable influence on prostaglandin stability. Our proposed model system successfully reconciles apparently anomalous or contradictory reports regarding the effect of albumin on prostaglandin stability.

Albumin, in vivo or in vitro, can sequester certain physiologically relevant ligands (Peters, 1970, 1975; Anton & Solomon, 1973). The sequestration of free fatty acids by albumin typifies this phenomenon (Spector, 1975). Although the fatty acids have been studied extensively in this context, similar studies with the prostaglandins (PG),[1] an important class of fatty acid metabolites, are limited. Like the fatty acids, certain prostaglandins can bind to albumin (Unger, 1972; Raz, 1972 a–c; Attallah & Schussler, 1973; Gueriguian, 1975). Unlike fatty acids, most naturally occurring prostaglandins have a considerable potential for hydrolysis, dehydration, or isomerization, depending on their immediate environment. In view of our recent report that albumin altered the inherent stability of prostaglandin I_2 (Wynalda & Fitzpatrick, 1980), we examined the possibility that albumin might expose other prostaglandins to unique environments that could influence their stability. An experimental survey shows that vertebrate albumins amplified the degradation of all naturally occurring primary prostaglandins containing a β-hydroxy ketone. The data permit a description of the environment that prostaglandins encounter when they interact with albumins. Furthermore, the finding that some, presumably stable, prostaglandins have significantly reduced stability in the presence of albumin may affect the design and interpretation of experiments in the field of eicosanoid research.

Experimental Procedures

Materials. Purified albumins were purchased from Sigma Chemical Co., St. Louis, MO, Calbiochem, La Jolla, CA, and Miles Biochemicals, Elkhart, IN. Crystallized and lyophilized human albumin [Sigma lot 108C-8085, Calbiochem lot 003097]; fraction V human albumin [Sigma lot 30F-02271, Calbiochem lots 903635 and 600638]; and essentially fatty acid free human albumin [Sigma lots 19C-7050 and 70F-9310, Miles Pentex lot 15] were used without further purification. Vertebrate albumins from rat [Sigma fraction V lots 30F-9330 and 30F-9331, Miles Pentex fraction V lot 28], dog [Sigma fraction V lots 96C-3963 and 30F-9305], pig [Sigma fraction V lot 60F-9355], cow [Sigma fatty acid free fraction V lot 10F-93371, Calbiochem fraction V lot 902295, Sigma fraction V lot 30F-9315], and rabbit [Sigma fraction V lot 47C-3965] were used without further purification. Prostaglandins (≥95% pure) were supplied by the Experimental Chemistry Laboratories of The Upjohn Co. Hexane, methylene chloride, acetonitrile, ethyl acetate, and methanol, all distilled in glass from Burdick & Jackson, formic acid, α-bromoacetonaphthone, and diisopropylethylamine from Aldrich Chemical, 1,3-butanediol from Eastman, and glyceryl guaiacolate from Sigma were used as received. Nucleosil 100–10 microparticulate silica gel and Nucleosil C18 microparticulate reversed-phase liquid chromatography columns were supplied by Rainen Instrument Co. Other reagents cited were of the highest quality commercially available.

Methods. Albumin solutions (1.0–20.0 mg/mL) were prepared with sterile 0.1 M, pH 7.4, potassium phosphate buffer containing 0.9% (w/v) NaCl and 0.01% (w/v) sodium azide. The pH remained at 7.4 ± 0.05 after the albumin dissolved. Prostaglandin solutions (100 μg/mL) were prepared by dissolving an appropriate prostaglandin (10.0 mg) in 100.0 mL of buffer or buffered albumin solution. The solutions were mixed by sonication (1 min) and incubated at 37 ± 1 °C. Samples (3.0 mL) were withdrawn periodically and transferred to glass tubes (15 mL) containing saturated ammonium sulfate (2.0 mL) and formic acid (20 μL) to monitor prostaglandin decomposition kinetics. The samples were extracted imme-

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[1] Abbreviations used: PG, prostaglandins; PGE_1, 11α,15α-dihydroxy-9-keto-5-*cis*-prostenoic acid; PGE_2, 11α,15α-dihydroxy-9-keto-5-*cis*,13-*trans*-prostadienoic acid; 6-keto-PGE_1, 11α,15α-dihydroxy-6,9-diketo-13-*trans*-prostenoic acid; 15-keto-PGE_2, 11α-hydroxy-9,15-diketo-5-*cis*,13-*trans*-prostadienoic acid; PGD_2, 9α,15α-dihydroxy-11-keto-5-*cis*,13-*trans*-prostadienoic acid; PGA_1, 15α-hydroxy-9-keto-10-*cis*,13-*trans*-prostadienoic acid; PGB_1, 15α-hydroxy-9-keto-8,13-*trans*-prostadienoic acid; HPLC, high-performance liquid chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; FFA, free fatty acids; HSA, human serum albumin.

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diately with ethyl acetate (3 × 5.0 mL), and the pooled organic layers were evaporated prior to quantitative analysis for their prostaglandin content. The extraction efficiency of PGE_1, PGE_2, 6-keto-PGE_1, 15-keto-PGE_2, and PGD_2 from all "time zero" samples was 97 ± 3%.

Derivatization. Prostaglandins were converted into naphthacyl esters for quantitative analysis by high-performance liquid chromatography (HPLC) with ultraviolet spectrophotometric detection (Cooper & Anders, 1974; Morozowich & Douglas, 1975). Briefly, the residue from the extraction was derivatized with α-bromoacetonaphthone (10 mg) and diisopropylethylamine (2 μL) in acetonitrile (1.0 mL). After complete reaction 1 h, 25 °C), the acetonitrile was evaporated. Prostaglandin naphthacyl esters were reconstituted in 5.0 mL of chromatographic mobile phase containing glyceryl guaiacolate (5.00 mg/mL) as an internal standard for quantitation. Adsorption chromatography on a microparticulate silica gel column 250 × 4 mm i.d.) with the mobile phase methylene chloride/1,3-butanediol/methanol/water (3970:24:3:3 v:v:v:v) (1.5 mL/min, 1000 psig) separated the naphthacyl esters of PGE_1, PGE_2, PGE_3, or 6-keto-PGE_1 from the naphthacyl esters of their respective isomerization and dehydration products. Adsorption chromatography with the mobile phase hexane/methylene chloride/1,3-butanediol/methanol/water (2000:1985:12:1.5:1.5 v:v:v:v:v) (2.0 mL/min, 1200 psig) separated the naphthacyl esters of 15-keto-PGE_2, PGD_1, or PGD_2 from the naphthacyl esters of their respective isomerization and dehydration products. For all assays, injections (20 μL) through a sampling valve were precise to ±1.5% relative standard deviation (n = 6). Chromatographic profiles were monitored by ultraviolet absorption at 254 nm. Quantitation was based on the peak height of the prostaglandin relative to the glyceryl guaiacolate internal standard.

In selected cases for each prostaglandin, we confirmed the adsorption chromatography results by a completely independent analysis using reversed-phase (RP) HPLC. Samples were prepared, incubated, extracted and derivatized as before but analyzed by RP-HPLC (Fitzpatrick, 1976a,b). We performed all studies with nonradioactive prostaglandins to avoid radiolysis as a factor contributing to the chemical decomposition of the prostaglandins. Conventional HPLC apparatus with a 254-nm UV detector is suitable to reproduce our experiments.

Determination of Decomposition Products. We monitored the products formed during the incubation of PGE_1 in buffer alone and in buffer containing albumin. Reversed-phase HPLC on a Nucleosil C18 column with the mobile phase acetonitrile/water/acetic acid (350:650:1) separated PGE_1, PGA_1, and PGB_1. Following extraction and reconstitution in methanol (1.0 mL), samples (20 μL) were injected without derivatization. PGA_1 was detected by its characteristic UV absorption at 220 nm; PGB_1 was detected by its characteristic UV absorption at 280 nm.

Results

At 37 °C in pH 7.4 phosphate buffer, PGE_1, 6-keto-PGE_1, PGE_2, 15-keto-PGE_2, and PGD_2, at initial concentrations of 100 μg/mL each, decomposed by first-order reaction kinetics with respective half-lives of 62.0 ± 3.9, 64.7 ± 4.0, 56.7 ± 3.3, 38.2 ± 3.5, and 12.1 ± 1.7 h. Addition of human albumin to otherwise identical buffer solutions accelerated the first-order decomposition kinetics of these prostaglandins (Table I). The severity of the acceleration depended on the albumin concentration from 1.0 to 20.0 mg/mL; however, it was independent of the source (Sigma, Calbiochem, and Miles), the type (crystalline, fraction V), or the free fatty acid content

Table I: Statistical Summary of Human Albumin Influences on Prostaglandin Stability

prostaglandin	albumin concn (mg/mL)	half-life (h) mean ± SD	N_{exptl}[a]	$N_{albumins}$[a]
PGE_1	0	61.97 ± 3.88	4	
	2.5	42.10 ± 1.79	4	3
	5.0	30.84 ± 3.20	5	4
	10.0	20.17 ± 3.62	4	2
	20.0	13.9	1	1
PGE_2	0	56.74 ± 3.30	6	
	1	47.02 ± 0.22	2	2
	2.5	31.84 ± 2.36	4	3
	5.0	22.50 ± 2.83	5	4
	10.0	11.29 ± 0.99	6	4
	20.0	7.49 ± 0.77	2	2
6-keto-PGE_1	0	64.73 ± 4.02	7	
	1	48.70	1	1
	2.5	44.40 ± 4.44	6	4
	5.0	35.61 ± 2.80	6	4
	10.0	24.44 ± 2.11	6	5
	20.0	14.53	1	1
15-keto-PGE_2	0	38.24 ± 3.50	6	
	2.5	18.67 ± 1.15	6	5
	5.0	11.09 ± 1.86	6	4
	10.0	5.31 ± 0.60	5	5
	20.0	3.55 ± 0.05	2	2
PGD_2	0	12.12 ± 1.67	8	
	1	8.70	1	1
	2.5	5.49 ± 0.42	5	4
	5.0	3.65 ± 0.32	7	5
	10.0	2.74 ± 0.41	6	3

[a] N_{exptl} = total number of experiments; $N_{albumins}$ = total number of different types and sources of albumins included under N_{exptl}.

(0.05–3.0 mol of free fatty acid/mol of albumin) of the human albumin used. First-order decomposition kinetics with correlation coefficients r^2 = 0.99 were maintained for every albumin tested. The data in Figure 1 from representative experiments with PGE_1 typify the results from all experiments with other prostaglandins.

On the basis of their half-lives in buffer alone, the prostaglandins were stable in the order 6-keto-PGE_1 ≥ PGE_1 > PGE_2 > 15-keto-PGE_2 > PGD_2. A plot of (half-life in albumin)/(half-life in buffer) as a function of albumin concentration shows that these prostaglandins responded to albumin in the same order as their intrinsic stability (Figure 2). That is, 15-keto-PGE_2, a less stable compound, was most sensitive to the destructive influence of albumin while 6-keto-PGE_1, a more stable compound, was least sensitive to albumin. Expressed in another way, it required less albumin to degrade a less stable prostaglandin. For example, from Figure 2, we calculate that human albumin concentrations of 2.4, 3.4, 5.0, and 6.4 mg/mL, respectively, reduced the half-lives of 15-keto-PGE_2, PGE_2, PGE_1, and 6-keto-PGE_1 to 0.5 of their respective values in buffer.

The effect of other vertebrate albumins on prostaglandin stability varied according to the species. However, every vertebrate albumin that we tested accelerated prostaglandin decomposition relative to a buffer control. Figures 3 and 4 show typical results for PGE_2 and PGD_2. At 10 mg/mL, human ($t_{1/2}$ = 11.1 h), baboon ($t_{1/2}$ = 10.6 h), and pig ($t_{1/2}$ = 11.2 h) albumins had a comparably severe effect on PGE_2 stability, and bovine albumin ($t_{1/2}$ = 33.5 h) had the least effect, while dog ($t_{1/2}$ = 14.5 h), rat ($t_{1/2}$ = 14.4 h), and rabbit ($t_{1/2}$ = 25.0 h) albumins had intermediate effects. Decomposition occurred by first-order kinetics in all cases. Results were similar for PGD_2. At 37 °C, pH 7.4, in the presence of

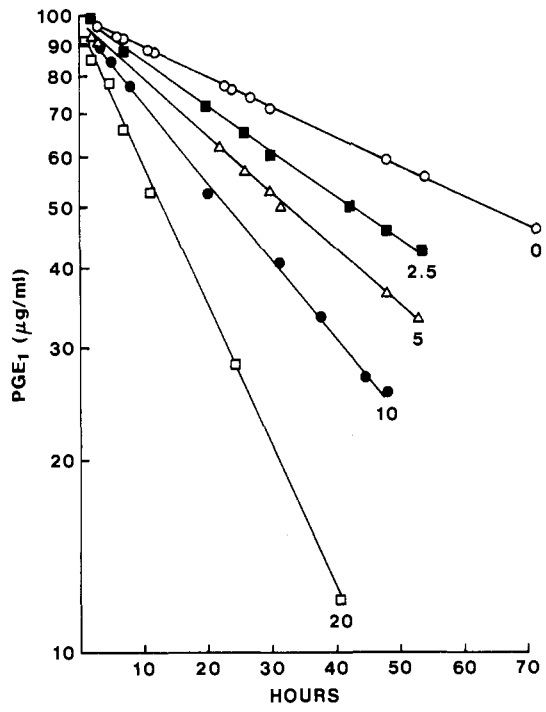

FIGURE 1: Influence of human albumin (0, 2.5, 5, 10, and 20 mg/mL) on the stability of PGE_1 (initial concentration 100 μg/mL) at 37 °C in 0.1 M, pH 7.4, potassium phosphate buffer containing 0.9% (w/v) NaCl and 0.01% NaN_3. Numbers in the figure indicate the albumin concentration. Samples (3.0 mL) were withdrawn at the times indicated, and PGE_1 was extracted and quantitated by high-performance liquid chromatography. Details are cited in the text.

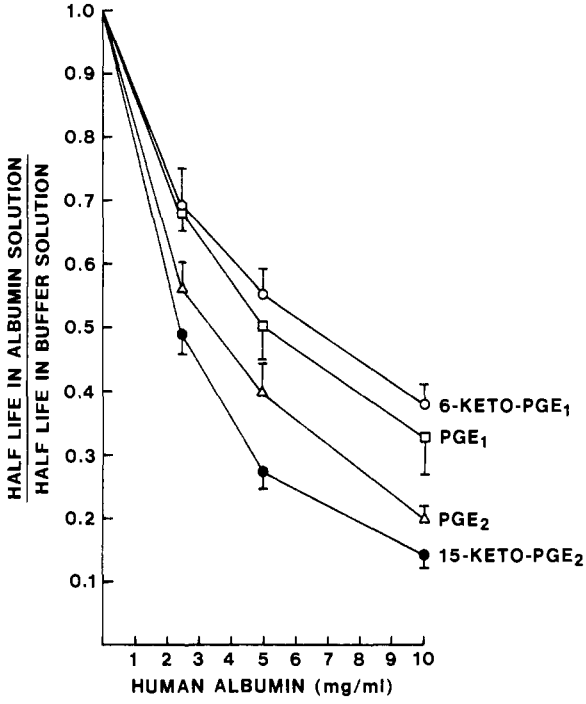

FIGURE 2: Relative stability [(half-life in albumin)/(half-life in buffer)] of E-type prostaglandins vs. human albumin concentration. The half-lives in buffer for 6-keto-PGE_1, PGE_1, PGE_2, and 15-keto-PGE_2 were 64.7 ± 4.0, 62.0 ± 3.9, 56.7 ± 3.3, and 38.2 ± 3.5 h, respectively.

10 mg/mL vertebrate albumins, the half-lives of PGD_2 were the following: human, $t_{1/2}$ = 2.7 h; pig, $t_{1/2}$ = 2.9 h; baboon, $t_{1/2}$ = 3.0 h; dog, $t_{1/2}$ = 3.8 h; rabbit, $t_{1/2}$ = 4.7 h; rat, $t_{1/2}$ = 5.2 h; and cow, $t_{1/2}$ = 6.8 h.

Albumins accelerated the decomposition of other typical prostaglandins (PGE_3, PGD_1, PGD_3, and 13,14-dihydro-15-keto-PGE_2) with a β-hydroxy ketone. In contrast, albumins had no destructive effect on several prostaglandins ($PGF_2\alpha$, $PGF_2\beta$, 13,14-dihydro-15-keto-$PGF_2\alpha$, and 6-keto-$PGF_1\alpha$) without a β-hydroxy ketone. Vertebrate globulins had no statistically significant influence on prostaglandin stability.

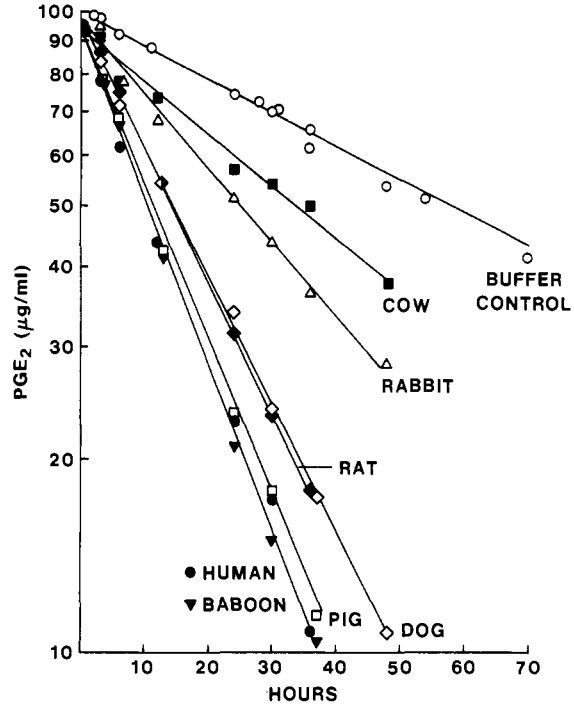

FIGURE 3: Influence of different vertebrate albumins (10 mg/mL) on the stability of PGE_2 (initial concentration, 100 μg/mL) at 37 °C in 0.1 M, pH 7.4, potassium phosphate buffer containing 0.9% (w/v) NaCl and 0.01% NaN_3.

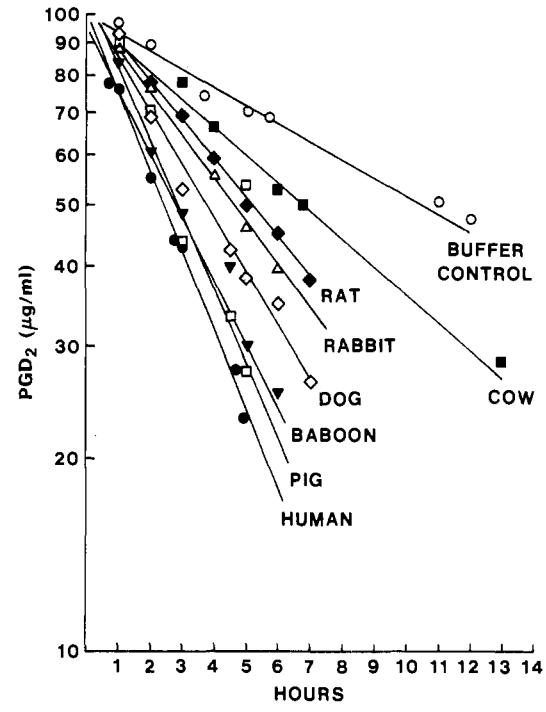

FIGURE 4: Influence of different vertebrate albumins (10 mg/mL) on the stability of PGD_2 (initial concentration, 100 μg/mL) at 37 °C in 0.1 M potassium phosphate buffer containing 0.9% (w/v) NaCl and 0.01% NaN_3.

Figure 5 shows that human albumin altered the qualitative aspects of prostaglandin decomposition. The left half of Figure 5 shows the chromatographic profile of decomposition products

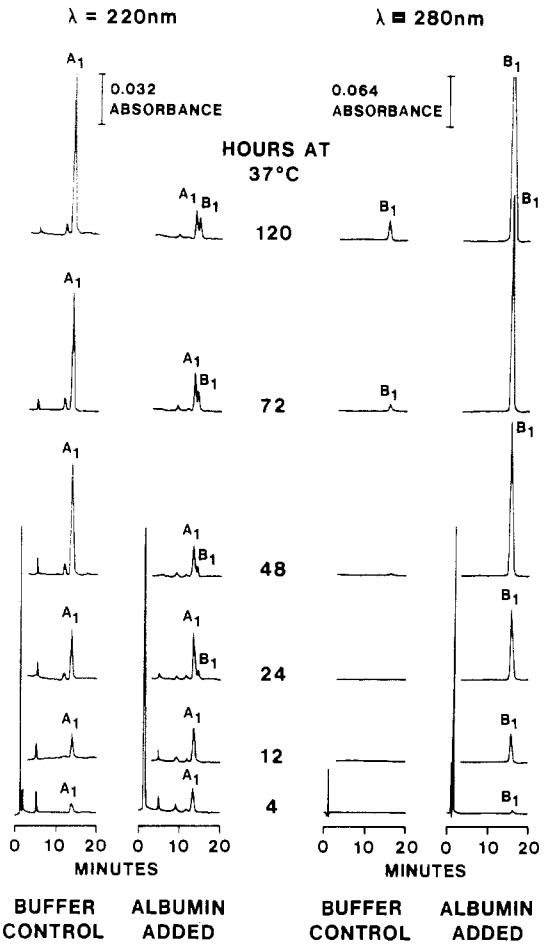

FIGURE 5: Influence of human albumin (20 mg/mL) on the degradation profile of PGE_1 (initial concentration 100 μg/mL) incubated at 37 °C in 0.1 M, pH 7.4, potassium phosphate buffer with 0.9% (w/v) NaCl and 0.01% (w/v) NaN_3. The left half of the figure uses UV monitoring at 220 nm for optimal detection of PGA_1. The right half of the figure uses UV monitoring at 280 nm for optimal detection of PGB_1. At each wavelength, buffer control is juxtaposed with buffer containing albumin (20 mg/mL). The vertical progression compares the decomposition profile from 4 to 120 h.

using UV detection at 220 nm. The right half of Figure 5 shows the profile with UV detection at 280 nm. The two main dehydration products of E-type prostaglandins are easily discriminated by their characteristic chromatographic mobility and UV absorption characteristics. PGA_1 with its 9-keto-Δ^{10} feature has a λ_{max} of 220 nm; PGB_1 with its Δ^8-9-keto feature has a λ_{max} of 280 nm. Using HPLC with UV detection at each wavelength, we compared the decomposition of PGE_1 (100 μg/mL) in 0.1 M, pH 7.4, buffer with its decomposition in an equivalent buffer containing albumin (20 mg/mL). In Figure 5, the profile with 220-nm detection shows that PGA_1 formation increased with time in the buffer control. In contrast, PGA_1 levels were nearly constant in buffer containing albumin, and at equivalent incubation times, they were typically less than the PGA_1 levels in the buffer control. Even though the extinction coefficient of PGB_1 is low at 220 nm, its formation was evident in the buffer containing albumin. The comparative HPLC profiles with 280-nm detection confirms this difference. In the buffer control, there was no PGB_1 detectable until 72 h, and it accounted for less than 10% of the total prostaglandins at 120 h. In contrast, with albumin added, PGB_1 appeared within 4 h, and it accounted for 85% of the total prostaglandins at 120 h. Besides PGA_1 and PGB_1, we identified 8-iso-PGE_1 (Daniels et al., 1968) and 11,15-dideoxy-10,12,14-prostatrienoic acid (Stehle & Oesterling, 1977) from samples containing albumin. Similar results were obtained for PGE_2 and 6-keto-PGE_1.

Discussion

Albumin accelerated the dehydration and alkaline-catalyzed isomerization of several structurally diverse, naturally occurring prostaglandins with a β-hydroxy ketone, regardless of their degree of saturation (PGE_1, PGE_2, and PGE_3), the orientation of their β-hydroxy ketone (E type, 9-keto-11α-hydroxy, vs. D type, 11-keto-9α-hydroxy, or the presence of different functional groups on their alkyl side chains (6-keto-PGE_1 and 15-keto-PGE_2). Evidently, albumin sequestered these prostaglandins and exposed them to a microenvironment at the protein surface that differed significantly from the solution macroenvironment. Because the destructive influence of albumin was nearly maximal at an initial mole ratio of 1:1 for albumin (20 mg/mL):prostaglandin (100 μg/mL), we conclude that human albumin mediates prostaglandin decomposition at one binding site and its associated microenvironment. An increase in the albumin:prostaglandin mole ratio beyond 1:1 had no further significant influence on prostaglandin stability at pH 7.4, 37 °C, while a reduction of the mole ratio to less than 1:1 attenuated the influence of albumin on prostaglandin stability. Upon their formation, the ultimate decomposition products, such as PGB_1, probably do not bind and occupy the destructive albumin microenvironment, or else they must be easily displaced by available prostaglandins with intact β-hydroxy ketones. If the decomposition products were themselves strongly bound, they would inhibit the binding and eventual destruction of intact prostaglandins whenever these were present in a molar excess relative to albumin. Such competitive inhibition is incompatible with the first-order reaction kinetics that were observed experimentally at 37 °C in every experiment.

All data indicated that albumin sequestration exposed the prostaglandins to an aqueous microenvironment with a pH of 10 or greater. For purely aqueous buffers, the pH-dependent decomposition of PGE_1 and PGE_2 has been thoroughly characterized (Monkhouse et al., 1973; Thompson et al., 1973; Stehle & Oesterling, 1977; Perera & Fedor, 1979). Dehydration occurs with accompanying formation of A- or B-type prostaglandins, mainly. Published pH vs. rate data show that dehydration occurs more slowly between pH 2.0 and 7.4 than at pH 7.4 (Monkhouse et al., 1973; Thompson et al., 1973). Below pH 2.0 or above pH 7.4, the dehydration rate increases relative to the rate at pH 7.4. According to our data, albumin accelerated the decomposition; this excludes microenvironments with a local pH between pH 2.0 and 7.4. Consequently, albumin must have exposed the prostaglandins to an aqueous microenvironment with a local pH either below 2 or greater than 7.4. Evidence favors a highly alkaline microenvironment with a local pH of 10 or greater. If albumin had exposed PGE_1 to an acidic microenvironment at pH ≤2, there would have been no formation of PGB_1 since isomerization of PGA_1 into PGB_1 is insignificant between pH 1 and 6 (Monkhouse et al., 1973; Perera & Fedor, 1979). Furthermore, in acidic microenvironments, allylic rearrangement of PGA_1 into 13-hydroxy-PGA_1 and 15-epiprostaglandins would dominate the decomposition profile of PGE_1 (Spraggins, 1972). In fact, PGB_1 was the dominant decomposition product. Both the rate and magnitude of its formation support the existence of an alkaline microenvironment with a local pH ≥10 since rapid isomerization occurs only under such alkaline conditions. Identification of minor decomposition products, 8-iso-PGE_1 and 11,15-dideoxy-9-keto-10,12,14-prostatrienoic acid, further supports a proposed alkaline microenvironment since the

formation of these compounds is incompatible with neutral or acidic conditions (Daniels et al., 1968; Stehle & Oesterling, 1977).

We recently reported that albumin altered the normal chemical behavior of two other prostaglandins (Fitzpatrick et al., 1980; Wynalda & Fitzpatrick, 1980). Consistent with our current survey, one of these compounds, 13,14-dihydro-15-keto-PGE_2, degraded more rapidly in the presence of albumin, and its degradation was accompanied by the appearance of an unique bicyclo rearrangement product that could only be formed under alkaline (pH ~10) conditions (Granstrom et al., 1980; Fitzpatrick et al., 1980). In contrast, PGI_2 degraded more slowly in neutral buffer containing albumin (Wynalda & Fitzpatrick, 1980). Our model for albumin–prostaglandin interactions does accommodate this, apparently, anomalous behavior of PGI_2. Instead of a β-hydroxy ketone, PGI_2 contains a vinyl ether group that is stabilized only under alkaline conditions (Cho & Allen, 1978; Chiang et al., 1979). According to our proposal, alkaline conditions exist near a prostaglandin sequestration site on albumin. Thus, one would expect albumin to stabilize a prostaglandin with an acid-sensitive vinyl ether, but degrade alkali-sensitive prostaglandins, like those with a β-hydroxy ketone. Without exception, to date the experimental evidence supports our conclusions.

Further experiments are needed to identify which amino acid residues of albumin are important, but it is notable that the lysine residue at position 199 is associated with acetylsalicylate binding and fatty acid binding (Hawkins et al., 1969; Pinckard et al., 1974), histidine at residue 146 is implicated in tryptophan binding (Lee & McMenamy, 1980), and lysine at residue 190 is implicated in pyridoxal 5′-phosphate binding (Gambhir et al., 1975). Furthermore, organic anion binding can occur at the sequence Lys-Ala-Trp-Ala-Val-Ala-Arg (Swaney & Klotz, 1970). The central hydrophobic zone flanked by lysine and arginine, with respective pK_a values of 10.5 and 12.5, is compatible with the requirements of our proposal. If Schiff-base formation with ϵ-amino groups of lysine was vital for the albumin effects, compounds with two carbonyls might behave differently than compounds with only one carbonyl; however, this was not evident. Both 6-keto-PGE_1 and 15-keto-PGE_2 were as sensitive to albumin as their monocarbonyl counterparts. We cannot exclude possible Schiff-base formation, but our results suggest that competition for available amine groups is independent of the carbonyl content of the naturally occurring prostaglandins tested.

As a protein class the albumins contain approximately twice the amount of basic amino acids (lysine, arginine, histidine) typical to other protein classes (Peters, 1970). If these basic amino acids are vital for the albumin effect, their location, their kind, and their arrangement must be more critical than their number, since the species differences in our results do not correlate readily with their amino acid content.

In vivo, plasma contains and albumin transports various endogenous and exogenous factors that are poorly soluble in aqueous media. The free fatty acids are important in this context. In plasma, the mole ratio of free fatty acids/albumin ordinarily ranges from 0.5 to 2.0 (Spector et al., 1970). We found that the destructive influence of albumin, in vitro, was unchanged when its fatty acid content ranged from 0.05 to 3.0 mol of FFA per mol of albumin. Thus, albumin can influence prostaglandin stability in the presence of other lipid anions. Our results agree with reports that serum and plasma augment the isomerization of PGC into PGB; however, we suggest that albumin is responsible, not a specfic enzyme (Polet & Levine, 1975). The possibility that every albumin preparation we used contained similar amounts of this putative enzyme is remote.

The finding that several, presumably stable prostaglandins have significantly reduced stability in the presence of vertebrate albumins may have important ramifications. For example, different groups have disputed the effect of albumin on the pharmacological activity (Unger, 1972; Raz, 1972a–c; Brown & Collier, 1975) and the metabolism of certain prostaglandins (Raz, 1972a–c; McDonald-Gibson et al., 1972; McGiff et al., 1969; Hawkins et al., 1977). The influence of albumin on their stability may explain these controversies. Certainly, the reports that albumin has a high capacity for binding prostaglandins through conventional hydrophobic and electrostatic interactions deserve reexamination (Unger, 1973; Raz, 1972a–c; Gueriguian, 1976). Also it is not self-evident that the activities of PGE_1, PGE_2, 6-keto-PGE_1, and PGD_2 are due exclusively to these prostaglandins. Depending on the experimental conditions, their actual concentration may differ significantly from their initial concentration, and any biological or pharmacological effects may derive from the composite influence of an intact prostaglandin and its decomposition products. A-type prostaglandins, especially, can be biologically active (Honn et al., 1979; Santoro et al., 1979, 1980).

Our results imply that the potential instability of β-hydroxy ketone prostaglandins can aggravate the already formidable difficulties of prostaglandin analysis. These difficulties, typically, include extremely low concentrations, rapid metabolism, and susceptibility to artifactual formation (Granstrom & Samuelsson, 1978). Instability must be added to this list. Since any decomposition prior to analysis is entirely independent of the chosen assay procedure, the problem will affect all procedures to some degree.

In summary, vertebrate albumins have a uniform and predictable effect on prostaglandin stability, with potentially important, still unknown, physiological implications.

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Some Redox Properties of Myohemerythrin from Retractor Muscle of *Themiste zostericola*†

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ABSTRACT: Distinct semimetmyohemerythrin species are produced by one-electron oxidation of deoxymyohemerythrin and one-electron reduction of metmyohemerythrin. The former, $(semimetmyo)_O$, changes (≥90%) to the latter, $(semimetmyo)_R$, with $k = 1.0 \times 10^{-2}$ s^{-1}, $\Delta H^{\ddagger} = 15.1$ kcal mol^{-1}, and $\Delta S^{\ddagger} = -17$ eu. Oxidation of $(semimetmyo)_O$ by $Fe(CN)_6^{3-}$ rapidly produces an unstable metmyohemerythrin form which converts to the final metmyohemerythrin with $k = 4.6 \times 10^{-3}$ s^{-1}, $\Delta H^{\ddagger} = 16.8$ kcal mol^{-1}, and $\Delta S^{\ddagger} = -13$ eu. The two met forms react at the same rate with N_3^-, but the unstable form reacts very rapidly with $S_2O_4^{2-}$ in contrast to stable metmyohemerythrin. $(Semimetmyo)_R$ or a mixture of metmyohemerythrin and deoxymyohemerythrin equilibrate very slowly to a mixture containing all three species. The rate constants for disproportionation and comproportionation are 0.89 M^{-1} s^{-1} and 9.4 M^{-1} s^{-1}, respectively. EPR spectra near liquid He temperatures and optical absorption spectra have been used to characterize and measure the rates at 25 °C, pH 8.2, and $I = 0.15$ M. The comparative behavior of octameric and monomeric protein is discussed.

Hemerythrin (Hr)[1] is an easily isolable respiratory protein from marine worms, whose properties have been well characterized and for which a good deal of structural data is available (Hendrickson, 1978; Kurtz et al., 1977; Loehr & Loehr, 1979; Stenkamp & Jensen, 1979). Each subunit of hemerythrin contains two nonheme irons. The deoxy form contains both irons in oxidation state +2 and interacts reversibly with oxygen to give the oxy form in which the Fe/O_2 stoichiometry is 2:1. Both deoxy and oxy forms are easily oxidized to a met species containing irons only in the oxidation state +3. This is no longer O_2 sensitive but does react with a number of anions to form adducts with distinctive spectra (Keresztes-Nagy & Klotz, 1965; Meloon & Wilkins, 1976; Olivas et al., 1979). In a series of recent studies, two distinctive semimet forms have been characterized, namely, $(semimet)_O$ from one-electron oxidation of deoxyhemerythrin and $(semimet)_R$ from one-electron reduction of methemerythrin (Harrington et al., 1978; Babcock et al., 1980; Bradić et al., 1980; Muhoberac et al., 1980; Harrington & Wilkins, 1981). These semimet species undergo a number of reactions which arise from the presence of both iron(II) and iron(III) in the molecule, including a remarkable intramolecular disproportionation (eq 1). Hemerythrin exists in a number of oligomeric forms—octameric in the coelomic fluid of a number of sipunculids (including *Phascolopsis gouldii*, *Themiste zostericola*, and *Themiste dyscritum*), trimeric in the coelomic

$$(Fe^{II}Fe^{III})_8 \rightleftarrows (Fe^{II}Fe^{II})_4(Fe^{III}Fe^{III})_4 \quad (1)$$

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[1] Abbreviations used: Hr, hemerythrin; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance.

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